SPINDLE DEVELOPMENT AND BEHAVIOR IN THE GIANT AMOEBA [1]

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The anaphase movement of chromosomes has long been a problem, many details of which are still unsolved. The elongation of the interzonal region of the spindle as first postulated by Belar, seems to play an important part in forcing groups of daughter chromosomes apart. The mechanism by which this elongation is accomplished has not been made clear. Evidence bearing on the question was presented by Short (1945), who found 20 anaphase and telophase spindles twisted to the right in the giant amoeba, when the groups of daughter chromosomes were over 17 microns apart. None were twisted to the left. "The constancy in the direction of the twisting would result from a property inherent in the spindle apparatus; possibly an uncoiling of spindle elements as elongation proceeds." Since no previous observations of interzonal twisting, constant in direction, have been recorded, and the observations of Short are limited to 21 spindles in one amoeba, it is important to determine whether the direction of twisting is always the same in different individuals and whether this twisting is always associated with elongation. Such determination may add much to our understanding of the mechanism of elongation and of the nature of the interzonal fibers as well.

The giant amoeba is favorable material for a study of the mitotic spindle. It is easily cultured in the laboratory and multiplies rapidly. It possesses a large number of nuclei, all of which divide at essentially the same time. These dividing nuclei are not confined within a limited space. The majority of divisions allow for spindle elongation without obstruction by a nearby wall or cell membrane. The living amoeba has a somewhat characteristic appearance at different mitotic stages, making it possible to select desired stages for study. The work reported here has been confined to those stages of mitosis in which the spindle is apparent, and has been concerned primarily with the direction and constancy of interzonal twisting.

Throughout this paper the term *interzonal* will be used when referring to that part of the spindle which is seen between the separating groups of daughter chromosomes. This part may be made up of continuous fibers as well. The term chromosomal fibers has been applied to that part of the spindle extending from

[1] The proper name of this organism has been the subject of considerable controversy for a number of years. It is variously known as *Chaos chaos*, *Pelomyxa carolinensis*, and *Amoeba carolinensis*. Recent discussions of the validity of the various names have been presented by Short (1946), Kudo (1946), and Wilson (1947). Authorities for the taxonomic names are found in these papers.

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each group of daughter chromosomes toward the polar region. The direction of twisting of the interzonal spindle has been referred to as "right-hand" or "to the right." This is intended to mean that if one observes a *side-view* of an interzonal spindle (as seen from either end, not to be confused with a polar-view) fibers at the uppermost focal level are seen to cross from the observer's left side of the nearest chromosome plate, to the observer's right side of the more distant plate, while fibers at the lowermost focal level cross from the observer's right of the nearer plate to the observer's left of the distant plate.

Observations made during the course of this study show that "pushing" forces are involved in anaphase separation. The data obtained show that a right-hand twist of the interzonal spindle, accompanied by an apparent relaxation of the fibers as the separation of the groups of daughter chromosomes continues, consistently appears when these groups have moved 18 to 20 microns apart.

Material and Methods

The original stock culture was obtained from the General Biological Supply House, Chicago, Illinois, in December 1945. The amoebae were grown in butter dishes half-filled with wheat culture medium, containing paramecia, rotifers, bacterial gloea, numerous ciliates and water molds. No difficulty was experienced in maintaining cultures in vigorous condition at ordinary laboratory temperatures at any time of the year.

Specimens selected from stock cultures were fixed with Carnoy's fixative, and stained with either Delafield's or Heidenhain's haematoxylin. The amoebae were mounted in diaphane direct from absolute alcohol or cleared in xylene and mounted in balsam.

Amoebae undergoing nuclear division have considerable depth at all of the mitotic stages. When they are placed on a slide they tend to become flattened if the surrounding water is partially removed. Further flattening is obtained by dropping the fixative directly on the animal from a pipette. This flattening facilitates the process of staining and destaining, as well as later study. Since, however, the possibility exists that the cytoplasmic movements involved in the flattening process might cause distortion of the spindles, few slides were made by this method. The majority of the animals studied were either dropped directly into the fixative, or were flooded with fixative before a detectable change in shape occurred.

Development and Behavior of the Spindle

Mid-prophase

Spindle fibers first make their appearance during mid-prophase. At this time the nuclear membrane is intact, and the nucleus appears as a flattened sphere, measuring 22×19 microns. The chromosomes, which are numerous and small, have become loosely grouped on a plate measuring 18×14 microns in polar-view. The chromosomal fibers are fairly distinct, lying approximately at right angles to the chromosome plate, and measuring four microns in length.

Late-prophase

At a slightly later stage of prophase, first described for the giant amoeba by Kudo (1947), and similar to a stage described and pictured by Liesche (1938) for

Amoeba proteus, the chromosomes are well oriented on a plate measuring 13 × 11 microns, and the nuclear membrane is sharply contracted until it is in contact with the ends of the chromosomal fibers. The fibers are four to five microns long, and are definitely, although not greatly arched. The entire nucleus in edge-view appears as a slightly rounded rectangle. Kudo (1947) in his description of this stage states that "the spindle fibers undergo further development and extend to the nuclear membrane." This is not in agreement with the observations of the writer. The fibers do not appear to increase appreciably in length, but rather the nuclear membrane contracts until it is in contact with the fibers.

Metaphase

The metaphase figure presents much the same picture as the late prophase stage described above, but the nuclear membrane has now disappeared, and the chromosome plate is more compact, measuring 11 × 9 microns. The arching, or curvature, and length of the chromosomal fibers show no appreciable change.

Anaphase-telophase

In an unflattened amoeba containing many nuclei, the anaphase or telophase figures are found distributed throughout the animal. They are found at all focal levels with their long axes vertical, horizontal and tilted at various angles. There are significantly more side-views than polar-views, as might be expected, since the depth of the animal is less than its other dimensions, but in other respects the distribution of figures is a random one. In some regions few figures are seen, while in others many may be found in close proximity. In an amoeba 60 microns thick, and containing many food vacuoles, some anaphase or telophase figures will inevitably be partly obscured, and perhaps a few entirely hidden. The spindles of strictly polar-views were not possible to analyze, and the same was true of some part-polar-views and obscured side-views (Table I).

Many different degrees of daughter chromosome separation were studied, involving more than forty amoebae. Distances between daughter groups varied from less than one micron to sixty-five microns. The appearance of the figure changes considerably as the groups of daughter chromosomes move progressively farther apart. Early stages continue to show the arching of chromosomal fibers observed at late prophase and metaphase, but this curvature is gradually lost. The chromosomes of each daughter group become increasingly closely aggregated on the plates. As the plates decrease in diameter the chromosomal fibers become more closely packed and straight. The outside chromosomal fibers at late anaphase are inclined at a sixty degree angle to the plate. They do not change materially in length but remain four to five microns long throughout division.

Early anaphases show the interzonal fibers as straight lines from plate to plate. Later, however, when the chromosome plates have moved farther apart, there is a pronounced barreling of these fibers—the diameter of the interzonal spindle being considerably greater at its center than its diameter close to either plate.

The appearance of granules along the ends of the chromosomal fibers has been taken to be the beginning of telophase since it is along the region of these granules that the daughter nuclear membrane forms (Short, 1946). If this is used as a criterion for the beginning of telophase, then there is an overlapping of anaphase and telophase stages with respect to the length of interzonal fibers. Granules at the ends of chromosomal fibers and even developing nuclear membranes have been observed when the groups of daughter chromosomes were as little as 20 microns apart, while more often groups much farther apart show no evidence of granules or membrane. When the new membrane has completely formed, both chromosomal and interzonal fibers disappear.

The fibers of the interzonal spindles, which are barreled, at mid-anaphase lie approximately parallel to one another. This barreling of the interzonal spindle clearly indicates that its elongation is meeting with resistance. Occasionally figures are seen in which daughter groups of chromosomes have encountered obstacles to their movement. In one such instance two anaphase figures, elongating in the same plane, met end to end. The two sets of chromosomal fibers which collided were crumpled, and interzonal fibers of both figures were buckled. Similar effects were observed in cases in which one or both groups of chromosomal fibers encountered a food vacuole or the pellicle. It seems evident that the pushing forces of elongation continue even when further separation of groups of daughter chromosomes is prevented.

When interzonal spindles reach a length of 18 to 20 microns, barreling of the fibers disappears. The fibers now appear relaxed. Accompanying this apparent relaxation is a pronounced and consistent right-hand twisting of the interzonal spindle. In no amoeba studied at or beyond this stage, were the interzonal spindles either straight or twisted to the left (Table I). A very few spindles showed the

TABLE I

Frequency of interzonal spindle twisting in two amoebae

Distance between plates—microns	Number of figures analyzed			Number twisted to right	Number twisted to left
	No. 1	No. 2	Total		
1–14	0	0	0	0	0
15–17	1	1	2	2	0
18–22	6	47	53	53	0
23–27	16	19	35	35	0
28–32	13	2	15	15	0
33–37	8	1	9	9	0
38–42	6	1	7	7	0
43–47	1	0	1	1	0
Analyzed	51	71	122	122	0
Undetermined*	55	37	92		
Total	106	108	214		

* Polar, semi-polar, and 27 side-views partially obscured.

right-hand twist when only 15 microns long. These were in animals in which most spindles were considerably longer. Elongation of the spindle continues and the twisting persists. The longest interzonal spindle which could be analyzed measured 45 microns, and showed a distinct right-hand twist. Many spindles of greater length were observed, the longest measuring 65 microns. These invariably had a rope-like appearance, and individual fibers could not be distinguished.

Several amoebae which showed twisted spindles did not show enough figures for statistical study. Two amoebae were selected which contained a large number of figures which could be accurately analyzed. Data obtained from these two animals are presented in Table I. The two animals contained a total of 122 spindles which it was possible to anaylze. These varied in length from 15 to 45 microns. All of these spindles were twisted to the right.

Discussion

Kudo (1947) discusses the reports of Short (1945, 1946), Dawson, Kessler, and Silverstein (1935), Hinchey (1937), Liesche (1938) and Nozawa (1939) of twisting of interzonal spindle fibers at late anaphase in various amoebae. He offers the simple explanation that "the inter-plate fibers become twisted because of the violent boiling movement of the protoplasm. . . ." Unfortunately he seems to have missed the point stressed by Short that "the fact that the spindles consistently twist in one direction indicates that this twisting which accompanies spindle elongation is not a chance phenomenon brought about by cytoplasmic movement or other external factors operating on the spindle." The same reasoning that Kudo uses in criticizing a statement of Hinchey would seem to apply in this case. In commenting on Hinchey's statement that "protoplasmic streaming next moves the plates apart," he makes the well taken point that "if this is the case, the streaming must necessarily be localized and the direction of streaming must be opposite for each of the hundreds of dividing nuclei, which cannot be verified." It seems equally unreasonable to suppose that "boiling" protoplasm could produce a twisting of spindles constant in direction.

Dawson, Kessler and Silverstein (1935) report spindle twisting in *Amoeba dubia* at mid-telophase. They state that "almost invariably a twisting of the spindle occurs at this stage." These workers unfortunately do not comment on the direction of twisting, but it is interesting to note that their illustration entitled "Typical twisting of spindle cylinder" shows a right-hand twist.

There are a number of striking similarities between the appearance of the spindle at various stages of division in the giant amoeba, and those described for the uninucleate *Amoeba fluvialis* by Dobell (1914). The chromosomal fibers of both animals are short, become more closely packed as division progresses, and do not change appreciably in length throughout division. Interzonal fibers of both animals are straight at early anaphase, barrel out as anaphase progresses and appear to relax at late anaphase or early telophase. It is noteworthy in this connection that while Dobell merely states that the spindles at this stage are "often somewhat twisted," his figure illustrating the stage likewise shows a distinct right-hand twist.

Hinchey (1937), in describing anaphase movement in the giant amoeba, states that "the chromatin then separates into two plates which move along the spindle fibers toward the poles until the plates are three times their diameter apart." It seems difficult to explain how the chromosome plates can "move along the spindle fibers." The writer is in agreement with the following statement of Dobell (1914) in reference to *Amoeba fluvialis*. "It seems certain that the daughter groups of chromosomes are separated from one another by the growth of the spindle fibers lying between them—not by the chromosomes moving along the spindle fibers; for the cap-like ends of the spindle remain of the same size during the whole process, whilst the fibers between the chromosome groups become longer and longer." Such a pushing apart of the groups of daughter chromosomes by the elongation of the interzonal fibers seems the only logical conclusion to be drawn from the barreling of these fibers at mid-anaphase and their buckling when obstacles to elongation are encountered.

When the extreme elongation of the interzonal fibers is considered it seems improbable that they arise from a stretching of the chromosome pellicle or sheath, as has been suggested by various workers on other material (Schrader, 1944). The evidence here seems to be all in support of a "pushing" hypothesis. It is suggested that the development of a definite fiber between centromeres, or the poles, might bring about the separation of the metaphase chromatids and that its continued elongation might account for the further separation on the part of the daughter chromosomes.

The constancy of the direction of the twist of the interzonal spindle must be determined by the molecular structure of the fibers. The molecular organization of these fibers may well be in the form of a coil, which when uncoiled would permit a twist in only one direction, accompanied by relaxation. The hypothesis of a molecular spiral has been proposed by Darlington (1935) to explain twisting of paired chromosomes about each other as they elongate at meiotic prophase. If lateral attractions between interzonal fibers exist, then the principles involved in Darlington's hypothesis for chromosomes would apply to the twisting of the spindles.

The work of Dawson, Dobell and other investigators mentioned above suggests that twisting of the interzonal spindle might be found to be a consistent phenomenon in species other than the giant amoeba. Amoebae are considered to be especially favorable material for a study of this phenomenon. Spindles of many organisms, being confined by small cell limits, may never reach a length that allows twisting to become apparent. A greater range of organisms, in which a considerable elongation of the spindle is possible, should be investigated for evidence of the twisting here described.

Summary

The chromosomal fibers of the giant amoeba appear during prophase, approximately at right angles to a loosely organized chromosome plate. At late prophase they are more closely grouped and slightly arched. This arching is observed during metaphase and early anaphase, the fibers becoming straight at mid-anaphase. They do not change materially in length at any stage of nuclear division. Chromosomal fibers become increasingly closely packed as the diameter of the chromosome plates decreases.

Interzonal spindles are straight at early anaphase, barrel out at mid-anaphase, and appear relaxed at late anaphase and telophase. They reach a length of 65 microns, but spindles more than 45 microns long appear rope-like and individual fibers cannot be distinguished. When groups of daughter chromosomes with their chromosomal fibers encounter an obstacle to their movement, chromosomal fibers are crumpled, and interzonal fibers buckle. This behavior, and the barreling of mid-anaphase spindles, must be interpreted as evidence that anaphase separa-

tion in this organism consists of a pushing apart of the groups of daughter chromosomes by the elongation of the interzonal spindle.

Interzonal spindles 18 or more microns long are consistently twisted to the right. Uncoiling of a spiral of constant direction within the interzonal fiber, accompanied by a lateral attraction between fibers, is offered as a possible explanation of the constancy of the direction of the twist.

LITERATURE CITED

Darlington, C. D., 1935. The internal mechanism of the chromosomes. *Proceedings of the Royal Society of London, Series B*, No. 807, **118**: 33–96.

Dawson, J. A., W. R. Kessler, and J. K. Silverstein, 1935. Mitosis in Amoeba dubia. *Biol. Bull.*, **69**: 447–461.

Dobell, C., 1914. Cytological studies on three species of amoeba—A. lacertae Hartmann, A. glebae n. sp., A. fluvialis n. sp. *Arch. Protistenk.*, **34**: 139–189.

Hinchey, M. Catherine, 1937. Mitosis in the giant amoeba Chaos chaos Linnaeus. *Biol. Bull.*, **73**: 368–369.

Kudo, R. R., 1946. Pelomyxa carolinensis Wilson. I. General observation on the Illinois stock. *Jour. Morph.*, **78**: No. 3, 317–352.

Kudo, R. R., 1947. Pelomyxa carolinensis Wilson. II. Nuclear division and plasmotomy. *Jour. Morph.*, **80**: 93–143.

Liesche, W., 1938. Die Kern- und Fortpflanzungsverhaltnisse von Amoeba proteus (Pall.). *Arch. Protistenk.*, **91**: 135–186.

Schrader, P., 1944. *Mitosis.* Columbia University Press.

Short, R. B., 1945. Spindle twisting in the giant amoeba. *Science*, **102**: No. 2654, page 484.

Short, R. B., 1946. Observations on the giant amoeba, Amoeba carolinensis (Wilson, 1900). *Biol. Bull.*, **90**: No. 1, 8–18.

Wilson, C. E., 1947. Concerning the correct name of the Rhizopod, Pelomyxa carolinensis. *Trans. Amer. Micr. Soc.*, **66**: 99–101.